REMARKS

Claims 1-59 are pending in the present application. Claims 4-8, 10, 11, 15-31, 33, 42-44, 46, 51, 55, 56 and 58 have been withdrawn from consideration. Reconsideration of the application is respectfully requested.

In the Office Action, claims 1-3, 9, 12-14, 32, 34-41, 45, 47-48, 52-54, 57 and 59 were rejected under 35 U.S.C. § 102 as allegedly being anticipated by Garretson (U.S. Patent No. 3,835,860). Claims 49 and 50 were rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Garretson. Applicants respectfully traverse the Examiner's rejections.

As the Examiner well knows, an anticipating reference by definition must disclose every limitation of the rejected claim in the same relationship to one another as set forth in the claim. *In re Bond*, 15 U.S.P.Q.2d 1566, 1567 (Fed. Cir. 1990). To the extent the Examiner relies on principles of inherency in making the anticipation rejections in the Office Action, inherency requires that the asserted proposition necessarily flow from the disclosure. *In re Oelrich*, 212 U.S.P.Q. 323, 326 (C.C.P.A. 1981); *Ex parte Levy*, 17 U.S.P.Q.2d 1461, 1463-64 (Bd. Pat. App. & Int. 1990); *Ex parte Skinner*, 2 U.S.P.Q.2d 1788, 1789 (Bd. Pat. App. & Int. 1987); *In re King*, 231 U.S.P.Q. 136, 138 (Fed. Cir. 1986). It is not enough that a reference could have, should have, or would have been used as the claimed invention. "The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *Oelrich*, at 326, quoting *Hansgirg v. Kemmer*, 40 U.S.P.Q. 665, 667 (C.C.P.A. 1939); *In re Rijckaert*, 28 U.S.P.Q.2d 1955, 1957 (Fed. Cir. 1993), quoting *Oelrich*, at 326; see also *Skinner*, at 1789. "Inherency ... may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." *Skinner*, at 1789, citing *Oelrich*. Where anticipation is found through inherency, the Office's burden of establishing prima facie

anticipation includes the burden of providing "...some evidence or scientific reasoning to establish the reasonableness of the examiner's belief that the functional limitation is an inherent characteristic of the prior art." *Skinner* at 1789.

Moreover, to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991); M.P.E.P. § 2142. Moreover, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (CCPA 1974). If an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988); M.P.E.P. § 2143.03.

With respect to alleged obviousness, there must be something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination. *Panduit Corp. v. Dennison Mfg. Co.*, 810 F.2d 1561 (Fed. Cir. 1986). In fact, the absence of a suggestion to combine is dispositive in an obviousness determination. *Gambro Lundia AB v. Baxter Health-care Corp.*, 110 F.3d 1573 (Fed. Cir. 1997). The mere fact that the prior art can be combined or modified does not make the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990); M.P.E.P. § 2143.01. The consistent criterion for determining obviousness is whether the prior

art would have suggested to one of ordinary skill in the art that the process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. Both the suggestion and the expectation of success must be founded in the prior art, not in the Applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991; *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988); M.P.E.P. § 2142.

In the present case, it appears that the Examiner's rejections are based, respectfully, on a misunderstanding of what is claimed. The present invention is directed to a device for forming holes in subchondral bone. The purpose of such holes is to induce fibrin clot formation and migration of primitive stem cells from the bone marrow into defective cartilage locations, to induce bleeding from the holes, and to release fat droplets from the holes. Specification, p. 4, ll. 12-21; p. 10, ll. 15-18; p. 12, ll. 10-11; p. 14, ll. 23-25; p. 15, l. 24 – p. 16, l. 2; p. 17, ll. 17-22.

Subchondral bone is bone that is "situated beneath cartilage." Merriam Webster's Medical Desk Dictionary (1986), p. 777 (copy attached). Also attached is a 1996 article entitled "Quantitative analysis of the bone-cartilage interface within the knee," describing aspects of subchondral bone associated with an illustrative knee joint. Of course, the present invention is not limited to use on subchondral bone associated with the human knee. The article is submitted to merely provide the Examiner with some background information.

With this understanding of what is being claimed in the present application, it is respectfully submitted that all pending claims are in condition for immediate allowance. Garretson is very far afield from the present invention. Garretson is understood to be directed to a surgical **bone punch** for use in forming small holes in the cranium that will match corresponding holes in a covering member so that an anchoring mechanism, *e.g.*, surgical wires, may be used to secure the covering member over an opening in the cranium. See, *e.g.*, Col. 1, ll.

26-31; Col. 2, ll. 47-52. Garretson does not contemplate the use of the device disclosed therein to form holes in subchondral bone – bone situated beneath cartilage.

In fact, it is not understood how the device in Garretson could be employed to form holes in subchondral bone. The device in Garretson comprises (see Figure 2) a punch guide 22, a bit 38 with a cutting end 39, and an anvil 52 formed at right angles to the path traveled by the bit 38. In use, bone, such as the cranium, is positioned between the anvil 52 and the base 48. Col. 4, ll. 7-14. Thereafter, the bit 38 is advanced forward by squeezing the trigger 27 multiple times. Each squeeze of the trigger 27 advances the bit 38 forward a distance that is substantially equal to the distance between the ratchet teeth 82. Col. 4, ll. 26-35.

It is simply not understood how the device disclosed in Garretson could be employed to form holes in subchondral bone. It is unclear how subchondral bone could be positioned between the base 48 and the anvil 52 of the device in Garretson.

Moreover, it is respectfully submitted that the present invention would not have been obvious in view of the prior art of record. A recent Federal Circuit case makes it crystal clear that, in an obviousness situation, the prior art must disclose each and every element of the claimed invention, and that any motivation to combine or modify the prior art must be based upon a suggestion in the prior art. *In re Lee*, 61 U.S.P.Q.2d 143 (Fed. Cir. 2002). Conclusory statements regarding common knowledge and common sense are insufficient to support a finding of obviousness. *Id.* at 1434-35. It is respectfully submitted that any attempt to assert that the inventions defined by the pending claims would have been obvious in view of the prior art of record would constitute an impermissible use of hindsight using Applicants' disclosure as a roadmap.

In view of the foregoing, it is respectfully submitted that all claims pending in the present application are in condition for immediate allowance. The Examiner is invited to contact the undersigned attorney at (713) 934-4055 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

WILLIAMS, MORGAN & AMERSON
CUSTOMER NO. 45488

Date: December 19, 2005

J. Mike Amerson
Reg. No. 35,426
10333 Richmond, Suite 1100
Houston, Texas 77042
(713) 934-4056
(713) 934-7011 (facsimile)

ATTORNEY FOR APPLICANTS

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sub•chon•dral \-'kän-drəl\ *adj* : situated beneath cartilage (~ bone)

sub•cho•roi•dal \ˌsəb-kɔ-'rȯid-ᵊl\ *adj* : situated or occurring between the choroid and the retina (~ fluid)

sub•class \'səb-ˌklas\ *n* : a category in biological classification ranking below a class and above an order

subclavia — see ARTERIA SUBCLAVIA

[1]**sub•cla•vi•an** \ˌsəb-'klā-vē-ən\ *adj* : of, relating to, being, or performed on a part (as an artery or vein) located under the clavicle (~ angioplasty)

[2]**subclavian** *n* : a subclavian part (as a subclavian artery or vein)

subclavian artery *n* : the proximal part of the main artery of the arm that arises on the right side from the brachiocephalic artery and on the left side from the arch of the aorta, that extends from its point of origin to the outer border of the first rib where it becomes the axillary artery and passes through the axilla and into the arm to become the brachial artery, and that supplies or gives off branches supplying the brain, neck, anterior wall of the thorax, and shoulder

subclavian trunk *n* : a large lymphatic vessel on each side of the body that receives lymph from the axilla and arms and that on the right side empties into the right lymphatic duct and on the left side into the thoracic duct

subclavian vein *n* : the proximal part of the main vein of the arm that is a continuation of the axillary vein and extends from the level of the first rib to the sternal end of the clavicle where it unites with the internal jugular vein to form the brachiocephalic vein

sub•cla•vi•us \ˌsəb-'klā-vē-əs\ *n, pl* **-vii** \-vē-ˌī\ : a small muscle on each side of the body that arises from the junction of the first rib and its cartilage, inserts into the inferior surface of the clavicle, and acts to stabilize the clavicle by depressing and drawing forward its lateral end during movements of the shoulder joint

sub•clin•i•cal \-'klin-i-kəl\ *adj* : not detectable or producing effects that are not detectable by the usual clinical tests (a ~ infection) (~ cancer) — **sub•clin•i•cal•ly** \-k(ə-)lē\ *adv*

sub•clone \'səb-ˌklōn\ *n* : a clone selected from a clone esp. after a mutation occurs (clones and ~s of human-mouse somatic cell hybrids were selected —T. B. Shows *et al*)

sub•co•ma \ˌsəb-'kō-mə\ *adj* : relating to, used in, or being (psychiatric therapy in which insulin is administered in doses too small to produce coma (~ insulin therapy)

sub•com•mis•sur•al organ \ˌsəb-ˌkäm-ə-ˌshûr-əl-\ *n* : an aggregation of columnar cells situated between the posterior commissure and the third ventricle of the brain

sub•con•junc•ti•val \ˌsəb-ˌkän-ˌjəŋ(k)-'tī-vəl\ *adj* : situated or occurring beneath the conjunctiva (~ hemorrhage) — **sub•con•junc•ti•val•ly** \-ē\ *adv*

sub•con•scious \ˌsəb-'kän-chəs, 'səb-\ *adj* 1 : existing in the mind but not immediately available to consciousness : affecting thought, feeling, and behavior without entering awareness (~ motives) (a ~ reflex) 2 : imperfectly conscious : partially but not fully aware (the persistence of ~ dream activity for several minutes after waking —*Psychological Abstracts*) — **sub•con•scious•ly** *adv* — **sub•con•scious•ness** *n*

subconscious *n* : the mental activities just below the threshold of consciousness; *also* : the aspect of the mind concerned with such activities — compare UNCONSCIOUS

sub•con•vul•sive \ˌsəb-kən-'vəl-siv\ *adj* 1 : inadequate to produce convulsions (~ doses of insulin) 2 : approaching the convulsive in character (a ~ reaction to noise)

sub•cor•a•coid \-'kȯr-ə-ˌkȯid, -'kȯr-\ *adj* : situated or occurring under the coracoid process of the scapula (a ~ dislocation of the humerus)

sub•cor•tex \ˌsəb-'kor-ˌteks, 'səb-\ *n* : the parts of the brain immediately beneath the cerebral cortex

sub•cor•ti•cal \-'kȯrt-i-kəl\ *adj* : of, relating to, involving, or being nerve centers below the cerebral cortex (~ lesions) (~ sensation) — **sub•cor•ti•cal•ly** \-i-k(ə-)lē\ *adv*

[1]**sub•cos•tal** \-'käs-təl\ *adj* : situated or performed below a rib (a left ~ incision)

[2]**subcostal** *n* : a subcostal part (as a muscle)

subcostal artery *n* : either of a pair of arteries that are the most posterior branches of the thoracic aorta and follow a course beneath the last pair of ribs

sub•cos•ta•lis \ˌsəb-käs-'tā-ləs\ *n, pl* **-ta•les** \-ˌlēz\ : any of a variable number of small muscles that arise on the inner surface of a rib, are inserted into the inner surface of the second or third rib below, and prob. function to draw adjacent ribs together

subcostal muscle *n* : SUBCOSTALIS

subcostal vein *n* : either of two veins: a : one that arises on the right side of the anterior abdominal wall, follows a course along the lower margin of the twelfth rib, and joins in the formation of the azygos vein — called also *right subcostal vein* b : one on the left side of the body that usually empties into the hemiazygos vein — called also *left subcostal vein*

sub•crep•i•tant \-'krep-ət-ənt\ *adj* : partially crepitant : faintly crepitant (~ rales)

[1]**sub•cul•ture** \'səb-ˌkəl-chər\ *n* 1 : a culture (as of bacteria) derived from another culture 2 : an act or instance of producing a subculture — **sub•cul•tur•al** \-'kəlch-(ə-)rəl\ *adj* — **sub•cul•tur•al•ly** \-ē\ *adv*

[2]**subculture** *vt* **-tured; -tur•ing** : to culture (as bacteria) anew on a fresh medium by inoculation from an older culture

sub•cu•ra•tive \ˌsəb-'kyůr-ət-iv\ *adj* : relating to or being a dose that is too small to produce a cure (~ amounts of one drug may become curative when given with another)

subcutanea — see TELA SUBCUTANEA

sub•cu•ta•ne•ous \ˌsəb-kyů-'tā-nē-əs\ *adj* : being, living, used, or made under the skin (~ parasites) — **sub•cu•ta•ne•ous•ly** *adv*

subcutaneous bursa *n* : a bursa lying between the skin and a bony process (as the olecranon of the elbow) or a ligament

subcutaneous emphysema *n* : the presence of a gas and esp. air in the subcutaneous tissue

sub•cu•tic•u•lar \-kyů-'tik-yə-lər\ *adj* : situated or occurring beneath a cuticle (~ sutures) (~ tissues)

sub•cu•tis \ˌsəb-'kyůt-əs, 'səb-\ *n* : the deeper part of the dermis

sub•del•toid \ˌsəb-'del-ˌtȯid\ *adj* : situated underneath or inferior to the deltoid muscle (~ calcareous deposits)

subdeltoid bursa *n* : the bursa that lies beneath the deltoid muscle and separates it from the capsule of the shoulder joint

sub•der•mal \-'dər-məl\ *adj* : SUBCUTANEOUS (a ~ injection) — **sub•der•mal•ly** \-ē\ *adv*

sub•di•a•phrag•mat•ic \ˌsəb-ˌdī-ə-frə(g)-'mat-ik, -ˌfrag-\ *adj* : situated, occurring, or performed below the diaphragm (a ~ abscess) (~ vagotomy)

sub•di•vi•sion \'səb-də-ˌvi-zhən\ *n* : a category in botanical classification ranking below a division and above a class

sub•du•ral \ˌsəb-'d(y)ůr-əl\ *adj* : situated, occurring, or performed under the dura mater or between the dura mater and the arachnoid (~ electrodes) (~ empyema) — **sub•du•ral•ly** \-ē\ *adv*

subdural hematoma *n* : a hematoma that occurs between the dura mater and arachnoid in the subdural space and that may apply neurologically significant pressure to the cerebral cortex

subdural space *n* : a fluid-filled space or potential space between the dura mater and the arachnoid

sub•en•do•car•di•al \ˌsəb-ˌen-dō-'kärd-ē-əl\ *adj* : situated or occurring beneath the endocardium or between the endocardium and myocardium (~ blood loss)

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THE KNEE

Quantitative analysis of the bone-cartilage interface within the knee

Barbara Koszyca[a], Nicola L. Fazzalari*[b], Barrie Vernon-Roberts[b]

[a] Department of Histopathology, Women's and Children's Hospital, 72 King William Road, Adelaide, SA 5006, Australia
[b] Division of Tissue Pathology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia

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Abstract

Changes in cartilage thickness, calcified cartilage thickness, subchondral bone plate thickness and cartilage vascularity have been proposed as mediators or initiators of degenerative joint disease, but the changes seen in these parameters in normal ageing have not been determined. Image analysis techniques were used to examine these parameters. Cartilage condition deteriorated significantly with age. Cartilage thickness decreased with age, most rapidly in the patella. Subchondral plate thickness was greatest in the medial tibial plateau, and decreased in thickness with age. Calcified cartilage thickness appeared as a constant, showing no regional variation nor any changes with age. This was despite the significant increase in tidemark numbers with age. It is hypothesized that there is an optimum thickness of calcified cartilage, which allows the firm anchoring of collagen fibres within the cartilage matrix without adversely affecting the elasticity of hyaline cartilage.

Keywords: Knee; Cartilage; Calcified cartilage; Tidemark; Subchondial bone

1. Introduction

The development of cartilage damage in synovial joints is a characteristic feature of osteoarthritis, but very similar, although less severe, changes are also seen in ageing joints. Thus it is important to have a clear understanding of the effects of ageing alone on the condition of a synovial joint, before speculating on the possible pathogenesis of osteoarthritis. There are a number of hypotheses concerning the initiation of cartilage damage in degenerative joint disease. It has been proposed that loss of proteoglycans from the matrix alone results in cartilage degeneration [1]; that decreased nutrition of the deeper layers of cartilage from subchondial vessels causes cartilage to become damaged [2–4]; and that increases in bone density render the overlying cartilage sensitive to loading forces [5]. In order to determine whether any of these changes could account for either the cartilage damage seen with increasing age, or for regional differences in cartilage damage, knee joints of individuals with no known history of joint disease were examined histologically. The parameters examined included total cartilage, calcified cartilage and subchondial bone thickness and subchondral vascularity. These factors were then related to overlying cartilage condition, to age and to regional differences in cartilage condition in an attempt to determine which factors best explained the observed pattern and nature of age-related cartilage changes within the knee.

2. Materials and methods

A total of 33 knee joints were obtained from 33 individuals, 15 female and 18 male, ranging in age from 18 to 90 years with a mean age of 61.81 ± 18.2 years. Excluded from the study were individuals known to have a history of knee disease, trauma or knee surgery or known to have metabolic bone disease or

* Corresponding author. Tel.: +61 8 2287269; fax: +61 82287204; email: nfazzala@immuno.imvs.sa.gov.au.

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disseminated malignancy. Specimens were obtained at autopsy by dissecting away the soft tissues, sectioning the femur and tibia at 7–10 cm from the tibiofemoral joint surface and disarticulating the tibiofibular joint. All specimens were immediately disarticulated and placed in 10% neutral formalin. Blocks for histology were taken from four regions of the knee: the medial tibial plateau, the medial femoral condyle, the patella and the trochlea (Fig. 1). The blocks were decalcified in a mixture of 1% ethylene diamine tetra-acetic acid and 9.5% nitric acid, before being processed into paraffin. Five-micrometre sections were cut, mounted and stained by the technique of Sayers et al. [6]. The condition of the hyaline cartilage was assessed using the Mankin score, a combination of histochemical and histological criteria [1]. This technique allows semi-quantitation of cartilage condition and is based on cartilage structure, cellularity, tidemark integrity and Safranin O staining: the higher the score the greater the degree of cartilage degeneration. In this study the Sayers technique [6] using Alcian blue, acid fuchsin and eosin was used instead of Safranin O. Sections stained by the Sayers technique provided clear delineation of the features at the bone-cartilage interface and were well adapted to the requirements of the image analyser.

The Quantimet Image Analysing computer 520 (Cambridge Instruments, Cambridge, UK) was used to measure a number of features at the bone-cartilage interface. The image was relayed from an Olympus BH2 microscope, via a video camera to the television screen of the Quantimet, and varying degrees of magnification were used to measure different features.

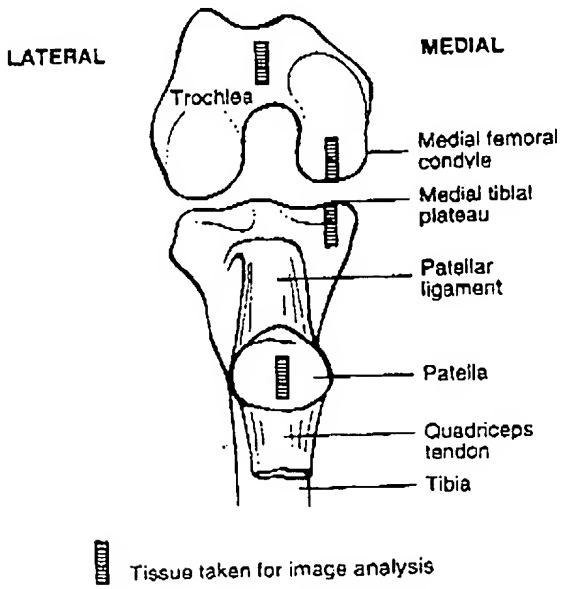

Fig. 1. Anterior aspect of the right knee with the patella reflected forward, indicating the sites from which tissue was taken.

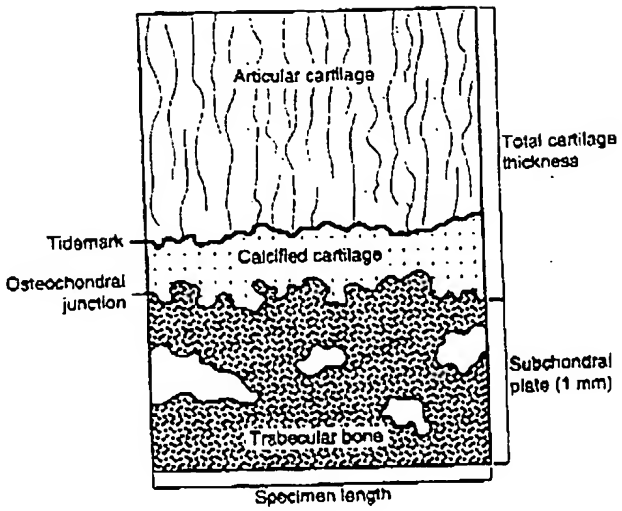

Fig. 2. Schematic diagram of the bone-cartilage interface.
Total cartilage thickness = Area of hyaline and calcified cartilage Specimen length
Subchondral plate thickness = Area of SCP 1 mm below the OCJ Specimen length
Subchondial plate = SCP
Osteochondral junction = OCJ
Calcified cartilage thickness = Area of calcified cartilage Specimen length.

Total cartilage thickness, comprising both hyaline and calcified cartilage was measured at 2 × objective magnification. The area of cartilage was outlined on the video screen using a cursor, the area determined, and the procedure repeated to include the entire section. The mean thickness was determined by dividing the area by the length of the section (Fig. 2).

The density and the thickness of the subchondial bone plate were measured to a depth of 1 mm below the osteochondial junction, from images at 4 × objective magnification. This method was chosen because of the poorly defined nature of the subchondial bone plate in the specimens studied.

Images at 10 × objective magnification were used to measure the mean thickness of the calcified cartilage layer (Fig. 2). The tidemark, or mineralisation front was seen as a pale line separating the calcified and the hyaline cartilage. In cases with multiple tidemarks the tidemark nearest the articular surface was used in determining the extent of the calcified cartilage layer. The maximum number of tidemarks seen in each block was noted as was the number of vascular contacts per 5 fields at 40 × objective magnification (Fig. 3). The vascular contacts consisted of vessels and surrounding soft tissue or woven bone, extending across the tidemark and providing a potential connection between the subchondral bone and the hyaline cartilage [7] (Fig. 4).

Data analyses for this study were conducted using PC-SAS software (SAS Institute Incorporated, Cary,

Fig. 3. Multiple tidemarks seen in an Alcian blue, acid fuchsin and eosin stained section from a patella bone block.

USA). Before any comparisons were made the nature of the distribution of each data group was determined using the Shapiro-Wilk statistic. Comparisons between groups were made using either paired or non-paired *t*-tests or the Wilcoxon rank statistic. Comparisons between more than two subgroups were conducted using a one-way analysis of variance or multiple Wilcoxon rank tests. Correlations were conducted using either the Pearson coefficient (r) or the Spearman rank coefficient (r_{rho}) of correlation. Linear regressions were conducted as indicated. The level of significance was taken to be $P < 0.05$ for all tests.

Fig. 4. Vascular contacts extending into and across the tidemark provide a potential connection between the subchondral bone and hyaline cartilage.

Table 1
Correlation of age (years) versus Mankin Score in the total study group (TSG) and in the four regions of the knee

Region	n	r	P	Correlation
TSG	132	0.484	< 0.0001	Mankin Score = 2.95 + 0.06 × Age
MF	33	0.296	NS	Mankin Score = 3.44 + 0.05 × Age
MT	33	0.504	< 0.01	Mankin Score = 3.44 + 0.05 × Age
TR	33	0.475	< 0.01	Manhin Score = 0.92 + 0.08 × Age
PA	33	0.437	< 0.02	Mankin Score = 4.00 + 0.05 × Age

NS, not significant; MF, medial femoral condyle; MT, medial tibial plateau; TR, Trochlea; PA, patella.

Table 2
Correlation of Mankin Score versus cartilage thickness (CT) in mm

Region	n	r	P	Correlation
TSG	132	−0.336	< 0.0001	CT = 3.5 − 145.2 × Mankin
MF	33	−0.429	< 0.02	CT = 3.0 − 140.3 × Mankin
MT	33	−0.243	NS	CT = 3.2 − 120.2 × Mankin
TR	33	−0.356	< 0.02	CT = 3.9 − 165.3 × Mankin
PA	33	−0.336	NS	CT = 4.1 − 174.8 × Mankin

NS, not significant; TSG, total study group; MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

3. Results

A total of 132 blocks were examined, from the medial femoral condyle, medial tibial plateau, trochlea and patella from the 33 individuals studied. There were no significant differences between male and female groups for any of the following parameters: hyaline cartilage thickness; calcified cartilage thickness; subchondral plate thickness and tidemark or vascular contact numbers ($P > 0.05$). There were no significant differences between specimens from right and left knees except for total cartilage thickness which was significantly greater on the right ($P < 0.02$).

Mankin score showed no significant variation between the four regions examined, but showed a significant positive correlation with age (Table 1). This correlation was significant in all four regions of the knee, except the medial femur (Table 1). A negative correlation was found between cartilage thickness and the Mankin score in the main study group and in both the medial femoral condyle and the trochlea (Table 2).

The number of tidemarks was significantly lower in the medial tibia than in the medial femur, trochlea or patella ($P < 0.001$) (Table 3). There was a significant positive correlation between age and the number of tidemarks ($r_{rho} = 0.353$, $P < 0.0001$, $n = 132$), but this was significant regionally only in the patella ($r_{rho} = 0.380$, $P < 0.05$, $n = 33$).

There were a significantly greater number of focal contacts in the trochlea than in the medial femur or medial tibia ($P < 0.02$) (Table 4). There was no significant correlation between focal contact numbers and age.

The total thickness of cartilage was significantly greater in the patella and trochlea than in either the medial femur or medial tibia ($P < 0.02$) (Table 5). A significant negative correlation was found between age and total cartilage thickness (Fig. 5). This correlation was significant in all regions examined except the medial femur (Table 6).

A significant positive correlation was found between total cartilage thickness (CT) and subchondral plate thickness ($n = 132$, $r = 0.292$, $P < 0.02$, SCP(mm) = 0.52 + 0.03 × CT). This correlation was not significant in any region alone.

No significant correlations were evident between

Table 3
Number of tidemarks from the four regions of the knee

Region	Minimum	25 percentile	Median	75 percentile	maximum
MF	1	2	3	4	7
MT	1	1	1	2	4
TR	1	2	3	4	6
PA	1	2	2	3	6

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

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Table 4
The number of focal contacts within the four regions of the knee

Region	Minimum	25 percentile	Median	75 percentile	Maximum
MF	1	5	8	11	17
MT	3	6	8	11	20
TR	5	8	11	12	18
PA	1	7	9	13	17

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

Table 5
Total cartilage thickness (mm) within the four regions examined

Region	Minimum	25 percentile	Median	75 percentile	Maximum
MF	0.93	1.39	2.23	2.73	3.41
MT	0.74	1.76	2.59	3.03	4.36
TR	1.07	1.77	3.00	4.01	4.93
PA	0.73	1.74	2.93	3.81	4.82

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

cartilage thickness and either the number of tidemarks or the number of focal contacts.

The thickness of the subchondral plate was significantly different in all four regions examined (Table 7) being greatest in the medial tibia ($P < 0.002$), then the patella ($P < 0.05$) and the trochlea ($P < 0.05$), and least in the medial femur ($P < 0.009$).

There was a significant negative correlation between age and subchondial plate thickness in the total study group (Fig. 6) which was significant in all regions except the trochlea (Table 8).

There were no significant differences in the thickness of calcified cartilage between the four regions examined (Table 9). There was no significant correlation between age and the thickness of calcified cartilage. No significant correlation was found between calcified cartilage thickness and any of the other parameters examined.

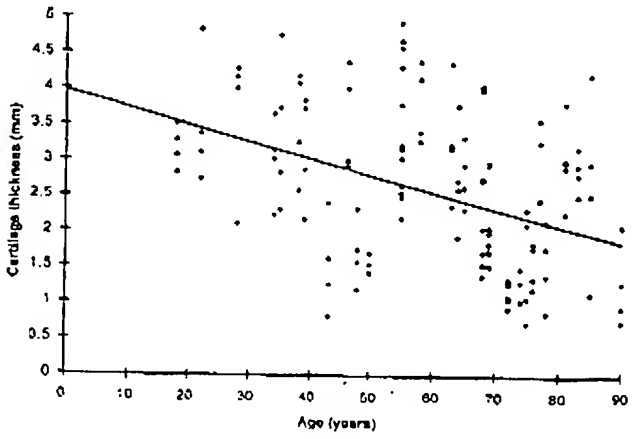

Fig. 5. Regression of total cartilage thickness (CT) on age (years) Total study group $n = 132$, $r = -0.411$, $P < 0.0001$, CT (mm) = 4.0 − 25.5 × Age.

4. Discussion

The lack of any regional difference in Mankin Score as found in this study is surprising since the patella is often more severely degenerated than the other regions of the knee, and bone exposure may develop in the patella independently of changes in the tibiofemoral compartment [2]. Examination of the correlations of age versus Mankin score (Table 1) shows that the patella had a higher score than the other regions at all ages, although this difference was not statistically significant. The rates of increase in Mankin score with age is similar in all regions suggesting that while the onset of cartilage changes is variable, once it is initiated it progresses at a similar rate in all regions. Thus, once cartilage is damaged, the progress of degeneration may be inexorable and unaffected by regional differences in functional demand.

Cartilage thickness is related to functional demand to the extent that areas with higher loading have thicker cartilage [8]. Cartilage plays an important role in the ability of a joint to deal with loading since it has a degree of elasticity by which potentially damaging forces can be absorbed [7]. Because it is able to deform when compressed, cartilage acts to spread loads over a larger area and thus minimise the compressive stresses in the underlying bone [5]. The thicker the cartilage is, the better able it is to deform with compression, and the larger the area over which loads are transmitted [9].

The mean value for cartilage thickness obtained in this study (2.12 ± 0.76 mm) is similar to that obtained by Stockwell [10] who found femoral condylar cartilage to be 2.26 ± 0.49 mm thick. Using the technique of stereophotogrammetry, similar values to those

Table 6
Correlation of age (years) versus total cartilage thickness (CT) in mm

Region	n	r	P	Correlation
MF	33	−0.312	NS	CT = 2.9 − 12.4 × Age
MT	33	−0.449	< 0.02	CT = 3.6 − 20.0 × Age
TR	33	−0.350	< 0.05	CT = 4.3 − 22.6 × Age
PA	33	−0.593	< 0.001	CT = 5.1 − 38.8 × Age

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

Table 7
Subchondral bone plate thickness (micrometres) within the four regions of the knee

Region	Minimum	25 percentile	Median	75 percentile	Maximum
MF	158	413	461	557	720
MT	472	677	720	792	904
TR	352	511	575	667	771
PA	501	573	637	683	807

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

found in this study for cartilage thicknesses have been found in the medial tibial facet, patella and femur [11]. These regional differences in cartilage thickness suggest that the functional demands on the patellofemoral joint, and particularly the trochlea, are greater than elsewhere in the knee. This is surprising since the loading forces per unit weight transmitted through the patellofemoral joint, during most normal activities, are lower than those transmitted through the medial compartment of the tibiofemoral joint [12]. The areas over which such forces are exerted are also similar: the contact area in the patellofemoral joint range from 2.95 to 5.00 cm^2 [13], and the mean contact area within the medial compartment of the tibiofemoral joint is 4.68 square cm [14]. Cartilage within the tibiofemoral joint is, however, protected by the presence of the menisci which transmit 45% of the load across the joint [12,15] and decrease the functional demands on the cartilage. This may contribute to the relative thinness of the cartilage on the medial femur and medial tibia.

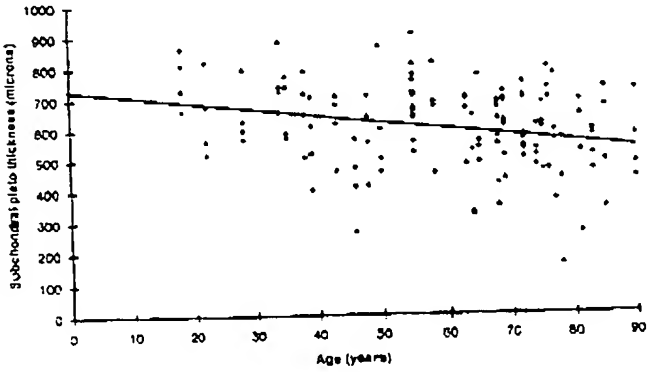

Fig. 6. Regression of subchondial plate thickness (SCP) on age (years) Total study group $n = 132$, $r = -0.298$, $P < 0.001$. SCP (micrometres) = 728 − 2.1 × Age.

Simon [16] found that cartilage thickness was not related to compressive forces across a joint but could not exclude the possibility that dynamic or non-compressive forces could influence cartilage thickness. If shear forces are important in determining cartilage thickness, the thickness of cartilage in the patella and trochlea can be explained by the greater proportion of shear forces to which the patellofemoral joint is exposed.

Table 8
Correlation between age (years) and subchondral bone plate thickness (micrometres)

Region	n	r	P	Correlation
MF	33	−0.464	< 0.01	SCP = 651 − 3.0 × Age
MT	33	−0.455	< 0.02	SCP = 860 − 2.4 × Age
TR	33	−0.2150	NS	SCP = 648 − 1.1 × Age
PA	33	−0.452	< 0.01	SCP = 751 − 2.0 × Age

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

Table 9
Calcified cartilage thickness (micrometres) within the four regions of the knee

Region	Minimum	25 percentile	Median	75 percentile	Maximum
MF	69	108	127	149	255
MT	77	117	137	168	242
TR	72	111	138	208	302
PA	88	113	132	170	264

MF, medial femoral condyle; MT, medial tibial plateau; TR, trochlea; PA, patella.

There is a significant decrease in cartilage thickness throughout the knee with increasing age, which takes place most rapidly in the patella. This correlation has been described before by Meachim et al. [17] who considered that such thinning was the result of degeneration and fibrillation, rather than a senescent shrinkage of otherwise normal cartilage. The decrease in cartilage thickness seen in this study is related to progressive fibrillation, described by the significant correlation found between cartilage thickness and Mankin score (Table 2). In a radiological study of cartilage thickness in the femoral condyles [18], it was found that the correlation between age and cartilage thickness was of only borderline significance, whereas, in the current study, 17% of the variability in cartilage thickness was accounted for by age. The difference in findings may well be related to the different techniques used, as the measurements in the study by Hall and Wyshak [18] were from X-rays with magnifications ranging from 25 to 40%.

Subchondral plate thickness decreases with age, and this may be part of the age-related bone loss seen in the trabecular network [19]. Subchondral plate thickness shows significant variation between the four regions examined and is thickest in the medial tibial plateau. The density of the subchondial plate in this region was also noted by Clark and Huber in their ultrastructural study of the bone-cartilage interface [20]. This thickness may reflect the need for added structural support to the mechanically weak concave surface where loading acts to separate the structural elements [21]. The thickness of the subchondral plate is significantly greater in the patella than in the trochlea, which is not directly weight bearing and does not require the bolstering of the subchondral plate seen in the tibia.

Subchondial plate thickness showed a significant positive correlation with total cartilage thickness. This is not surprising since both tissues are known to respond to functional demands [22,23], and both have been shown in this study to be significantly related to age. It is, however, interesting that the region with the thickest cartilage, the trochlea, is not the region with the thickest subchondial plate. This is to be expected since the two tissues have very different structure and function even though both have a role to play in the absorption of potentially damaging forces acting across a joint.

In this study, the thickness of calcified cartilage was found to range from 69 μm to 302 μm, with a mean value of 147 $\pm$ 52 μm. The mean thickness of calcified cartilage in the patella was 148 $\pm$ 50 μm. Previous studies have found the thickness of calcified cartilage to range from 20 to 230 μm in the femoral head [8] and to have a mean value of 134 μm in the patella [25]. In the current study, as in that of Green et al. [25], the thickness of calcified cartilage was not found to vary with age or to change with the condition of the hyaline layer. By contrast, Lane and Bullough [26], in a study of both femoral and humeral heads, found that there was a significant decrease in the thickness of the calcified layer in both males and females with increasing age. These anomalies may be related to differences in technique since Green et al. [25] used a microradiographic method, whilst Lane and Bullough [26] used histological techniques and measured the calcified layer thickness using a reference grid eyepiece. Measurements made by this latter method would be complicated by the very irregular border of the calcified layer where it adheres to bone. The Quantimet image analysis system used in the present study has undoubted technical advantages, being able to incorporate all elements of calcified cartilage within the section into the calculations of thickness and obtain a value more representative of the region examined. The study of Muller-Gerbl et al. [8], which utilised similar image analysis techniques to those used here, did not have a sufficiently large sample to determine the presence of any age-related change in calcified cartilage thickness. Previous studies have found the calcified layer to be thicker in stressed, as opposed to non-stressed, areas in the femoral head [2,15,27] but in the current study no regional variation could be seen, although comparisons here were made between different articular surfaces rather than different areas in the same joint. Milington [28] found that calcified cartilage thickness varied widely within one joint ranging from a few micrometres to 1.5 mm. Muller-Gerbl et al. [8] and Oegema [24] found that there was a significant correlation between calcified and non-calcified cartilage, and Muller-Gerbl et al. [8] noted the wide individual variability in each of the 8

subjects that they studied. These facts may explain the lack of any correlation between calcified and hyaline cartilage in the current study where there is pooled data from 4 regions of 33 subjects.

The increase in the number of tidemarks with age [25,29,30] has been confirmed. This increase has been suggested to reflect past episodes of calcification activity at the tidemark. If this is so, it would be expected that the thickness of the calcified cartilage would increase with age with recurrent episodes of calcification at the tidemark. As calcified cartilage thickness remains unchanged with age it would appear that, as calcification proceeds at the tidemark, calcified cartilage is concurrently remodelled, presumably by the action of the osteoclasts and chondroclasts within focal contacts. If the role of the calcified cartilage is to provide a transition zone between the cartilage and bone to enhance the binding between these areas [25,30], there may well be an optimal thickness. If the calcified cartilage is too thin it will be an insufficient anchor for the collagen fibres of the hyaline cartilage, and if it is too thick it will provide a barrier against cartilage nutrition or the hyaline cartilage will be rendered inelastic and will be unable to fulfil its role in the transmission and distribution of loading forces. This optimal thickness may be maintained by an equilibrium between calcification and resorption.

The number of tidemarks is significantly lower in the tibia than in the other regions studied. This is surprising if reactivation is thought to be a response to stresses experienced by the articular surface, since a similar pattern of loading would be expected in the tibia and in the opposing femoral condyle. The stresses may not be equivalent if the menisci act to diminish the load transferred directly to the cartilage surface. Alternatively, the number of tidemarks in the tibia may be similar to those seen in the femur but they are somehow obscured from view. This, however, seems unlikely. Recent work by Revell et al. [30] found that, in individuals with multiple tidemarks, more than one tidemark may be metabolically active at one time. This work was restricted to femoral heads affected by osteoarthritis and cannot be thought of as representing the normal state although this is possibly the case. The role of the tidemark and the method of duplication remain unclear.

Focal contacts were significantly more numerous in the trochlea than elsewhere, and, as this was also the region with the thickest cartilage, this would suggest that focal contacts are involved in the nutrition of cartilage [3,4,31]. There was, however, no significant correlation between cartilage thickness and focal contact numbers, nor between subchondral plate thickness and focal contact numbers; this suggests that a thick subchondral plate does not prevent blood vessels from reaching the deeper layers of cartilage, as has been proposed in some models of osteoarthritic cartilage damage [31].

The most striking feature of this study was the constancy of the calcified cartilage thickness in the knee. It does not vary with age or with region, unlike the total thickness of cartilage or the thickness of the subchondral plate. This suggests an active process to maintain the thickness of the calcified layer. The current study would indicate that the constancy of the calcified cartilage layer may be the result of the tidemark and focal contacts acting in a coordinated manner, the tidemark forming the calcified layer and the chondroclasts and osteoclasts within focal contacts resorbing the calcified cartilage. This process may be started by calcification at the tidemark in response to local factors, such as the products of cartilage fibrillation and breakdown, or substances produced by proliferating chondrocytes. Thickening of the calcified cartilage may result in increasing hypoxia in the deeper layers providing the stimulus for vascular formation and thus the infiltration of focal contacts may be stimulated. It can be hypothesized that the great variation and decrease in the calcified cartilage thickness described in osteoarthritis [28], may be the result of a disturbance in the equilibrium between the tidemark and the focal contacts. Such a decrease in calcified cartilage thickness would suggest excessive resorption of this layer, possibly as the result of increased vascularity secondary to increased remodelling of the bone.

In the current work the constant nature of calcified cartilage thickness argues against changes in this zone being significant in the development of cartilage damage in age-related changes, but does not exclude the possibility that remodelling of this area may have some role. The possibility that changes in the proposed calcified cartilage equilibrium may have a role in osteoarthritis requires extensive study of that calcified cartilage layer in diseased joints. Other possible mechanisms of cartilage damage that have been put forward, including changes in the cancellous bone, must be examined before the calcified layer can be considered to have the key to the initiation of cartilage damage and age-related changes in the knee.

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